

ALTERED HATCH SEQUENCE OF MALES AND FEMALES FROM  
UNCHILLED EGGS OF A "NON-DIAPAUSE" GYPSY MOTH  
STRAIN (LEPIDOPTERA: LYMANTRIIDAE)

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*Abstract.*—Larvae hatching from unchilled egg masses of a "non-diapause" gypsy moth strain (*Lymantria dispar* Linnaeus) were reared to determine the hatch sequence of males and females. Males tended to hatch from non-diapause egg masses before females, which is the reverse of the hatching sequence of chilled wild egg masses. These results were obtained with an outcrossed non-diapause strain as well. The reversed hatch sequence is not due to a skewed sex ratio or to differential mortality.

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The gypsy moth (*Lymantria dispar* Linnaeus) has been intensively studied in North America even though it has been a difficult laboratory animal. Development of an artificial diet (Leonard and Doane 1966; ODell and Rolinson 1966) and a "non-diapause" strain (Hoy 1977, 1978a, b) have made laboratory rearing easier. The non-diapause strain facilitates continuous laboratory rearing for parasite or virus production since the gypsy moth eggs will hatch within 31 days of deposition without exposure to cold. Wild type gypsy moth eggs require 90-120 days of chill to obtain a good level of hatch.

The non-diapause strain had been selected simultaneously for early hatch and hatch without chilling (Hoy 1977, 1978a, b). Beginning with generation 5, the first 60% of larvae to hatch from each egg mass were reared and larvae hatching later were discarded. In generation 6 the first 40% of larvae to hatch were reared, and in generation 7 only the first 20% to hatch were reared. At the same time, the sex ratio of the adults reared from these fractions of egg masses shifted; progressively higher frequencies of males were produced in each succeeding generation. This study was undertaken to determine whether the excessive number of males produced was due to a change in sex ratio in the non-diapause strain or to a change in the time of hatch of males and females in this strain. Accordingly, all larvae hatching from twenty-three unchilled non-diapause egg masses were reared.

## Materials and Methods

Intact, unsterilized egg masses were held individually in plastic petri dishes ( $100 \times 15$  mm). They were supplied with a block of synthetic diet (Media 722A, BioServ, Inc., Frenchtown, N.J.). Larvae began hatching about 30 days after egg deposition. Newly hatched larvae were collected daily from 10 non-diapause (selection generation 8 (Hoy 1977, 1978a)) egg masses. These larvae were reared in groups of 20 in 475 ml unwaxed cardboard containers supplied with diet in small cups. Egg masses and larvae were maintained at  $21\text{--}22^\circ\text{C}$  and 16 h light. Sex was determined by examining pupae.

To see if the apparently skewed sex ratio could be modified by outbreeding with a wild gypsy moth stock, an outcross was made of the selected non-diapause strain to adult moths reared from field-collected wild eggs. This was followed by a backcross of the  $F_1$  progeny to the non-diapause strain. The resulting strain was selected for "non-diapause" for 3 generations. Larvae from 13 egg masses of the 4th outcrossed non-diapause generation were group reared as above except larvae from 4 of the 13 egg masses were reared individually in  $100 \times 15$  mm petri dishes to reduce larval mortality due to disease.

## Results and Discussion

All three groups of egg masses had the same male-female hatch sequence (Fig. 1). High frequencies of male pupae were reared from the first collections and progressively lower proportions were reared from subsequent collections (Fig. 1). This hatch sequence is the reverse of that reported for chilled wild gypsy moth eggs. Leonard (1968) reported that the first larvae to hatch from field collected eggs consisted of ca. 30% males while the last larvae to hatch were ca. 65% males. We observed this wild-type hatching sequence repeatedly in our own laboratory (Hoy and Knop, unpubl. data). Mortality data are given in Fig. 2.

Greater susceptibility to mortality factors of females in first collections and males in later collections might produce a pupal (male-female) sequence such as we observed. Our data for the group with the lowest mortality (individually reared outcross non-diapause, Fig. 1, Fig. 2C) show that the male-female pupal sequence cannot be eliminated even if we include the dead larvae in the sex ratio calculations. Even if we assume that all the dead larvae in collections 1–5 were female and all the dead larvae in collections 10–14 (no. pupae  $> 10$ , Fig. 1) were male, collections 1–5 are more than 65% male and collections 10–14 are less than 40% male. Also, the number of male pupae in the first collection is significantly greater ( $\chi^2$ ,  $P < .001$ ) than the number of female pupae and dead larvae combined in

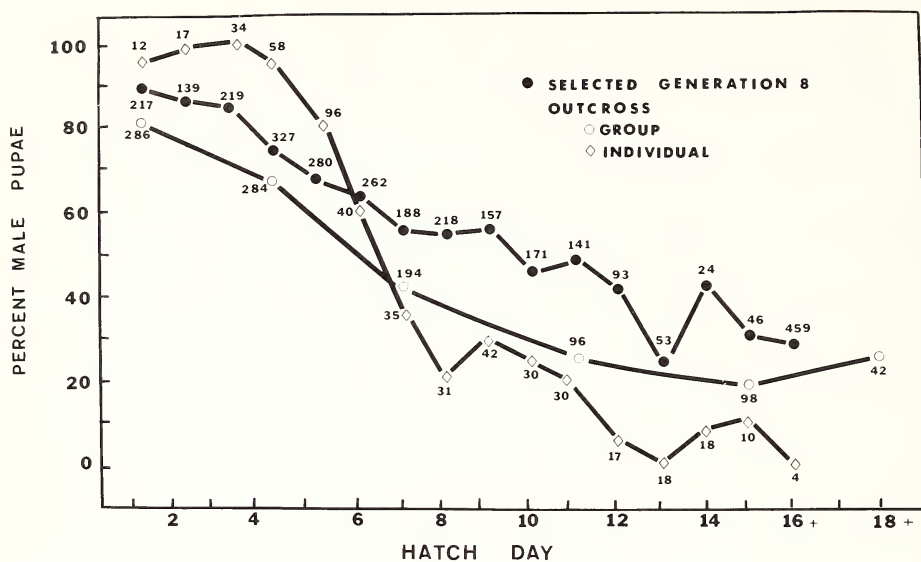


Fig. 1. Percentage male gypsy moth pupae reared from collections of larvae hatching from non-diapause (selected generation 8) and outcrossed non-diapause egg masses, group reared and individually reared. Numbers of pupae are indicated at each point.

each of the other groups (Fig. 1, Fig. 2A, B). We conclude that a male-female hatch sequence occurs in these non-diapause strains.

The overall sex ratios of the non-diapause and the outcrossed non-diapause strains are not different from the wild strain. Higher proportions of males (58–59%) were produced in group rearing but this was the result of higher mortality among late-hatching (mostly female) larvae (Fig. 2A, B). Individual rearing eliminated this skewed distribution of mortality (Fig. 2C) and pupae were 52% male.

Reversal in male-female hatching in the non-diapause gypsy moth strains is not due to a change in sex ratio or to differential mortality. We do not know if the reversal is a pleiotropic effect of the non-diapause trait, or is correlated with it through linkage or through correlated selection. Hoy (1978b) speculated that the "non-diapause" strain still has a short diapause, the selection having influenced diapause duration and the requirement for chilling. Wild egg masses that are chilled for less than the optimal time produce males first (Montgomery, unpubl. data). The occurrence and duration of chill may be important in determining hatch order in wild and "non-diapause" strains. (Chilled non-diapause egg masses hatch rapidly (Hoy 1978a) and any alteration in hatch order is obscured.)

The non-diapause gypsy moth strain was developed to facilitate contin-

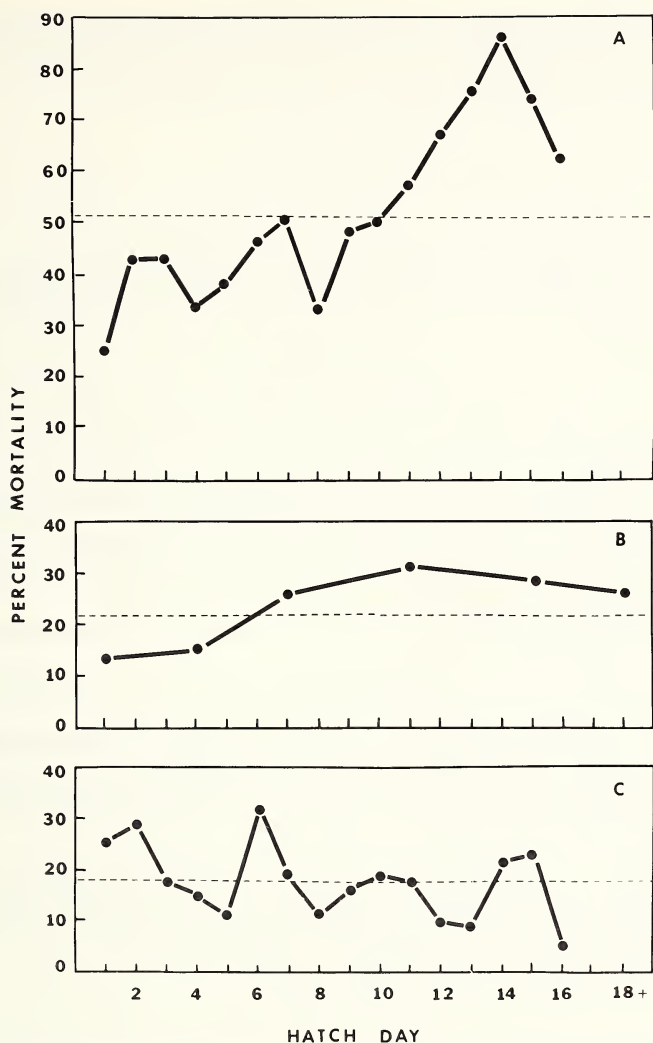


Fig. 2. Percentage developmental mortality in collections of gypsy moth larvae hatching from group reared non-diapause (selected generation 8) (A), and outcrossed non-diapause egg masses either group reared (B) or individually reared (C). The broken line indicates overall percent mortality.

uous rearing of gypsy moth parasites. The data presented here should facilitate maintenance of a desired sex ratio in larvae hatching from unchilled egg masses of this non-diapause strain. A higher proportion of adult females will result if the first non-diapause larvae to hatch are discarded. This would be advantageous if the caterpillars were being reared to yield nuclear-poly-

hedrosis virus since a higher yield is obtained from females than from males. Conversely, if sterile males were being reared for a genetic control program, it would be most profitable to rear only the first 30% of the larvae to hatch.

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